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ORIGINAL ARTICLE

Acoustic Characteristics of Advertisement Calls in *Babina adenopleura*

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Abstract Acoustic communication is the most important form of communication in anuran amphibians. To understand the acoustic characteristics of male *Babina adenopleura*, we recorded advertisement calls and analyzed their acoustic parameters during the breeding season. Male *B. adenopleura* produced calls with a variable number of notes (1–5), and each note contained harmonics. Although 6% of call notes did not exhibit frequency modulation (FM), two call note FM patterns were observed: (1) upward FM; (2) upward–downward FM. With the exception of 1- and 5- note calls, the duration of successive notes decreased monotonically. With the exception of 1 note calls, the fundamental frequency of the first note was lowest, then increased; the greatest change in the fundamental frequency was always between notes 1 and 2. The dominant frequency varied between calls. For example for the first call note the dominant frequency occurred in some cases in the first harmonic (located in the 605.320 ± 64.533 Hz frequency band), the second harmonic (918 ± 9 Hz band), the fourth harmonic (1712 ± 333 Hz band), the sixth harmonic (the 2165 ± 152 Hz band), the seventh harmonic (the 2269 ± 140 Hz band), the eighth harmonic (the 2466 ± 15 Hz band) or the ninth harmonic (the 2636 ± 21 Hz band). Although male *B. adenopleura* advertisement calls have a distinctive structure, they have similar characteristics to the calls of the music frog, *B. daunchina*.

Keywords anuran amphibians, advertisement calls, *Babina adenopleura*, call structure

1. Introduction

Vocalizations are one of the most important means of animal communication, playing a very important role in species identification and female mate choice in frogs and toads (Cunningham and Birkhead, 1998; Brenowitz and Rose, 1999). On the basis of their function and environmental context, four types of anuran calls have been categorized; reciprocation calls, release calls, distress calls and advertisement calls (courtship, territorial, and encounter calls) (Duellman and Trueb, 1986). Of these, advertisement calls have been the most extensively studied due to their unique species-specific characteristics (Gerhardt, 1994). During the breeding season, advertisement calls have a direct impact on individual reproductive success (Lingau and Bastos, 2007), and are regarded as one of the key characters responsible for reproductive isolation and speciation in anurans (Wells, 1977; Cocroft and Ryan, 1995; Pough *et al.*, 2004; Hernandez *et al.*, 2010; Bilate and Lack, 2011). In some species, such as *Duttaphrynus melanostictus* (synonym: *Bufo melanostictus*) (Wei *et al.*, 2012), male calls are relatively simple, consisting of a series of identical repeated notes, whereas in others, such as *Babina daunchina*, males can produce dozens of complex calls (Chen *et al.*, 2012). Due to this high level of species-specific variability, advertisement calls have been used to clarify the taxonomic relationships between closely related anuran species (Abrunhosa *et al.*, 2005; Bickford *et al.*, 2007; Nunes *et al.*, 2007; Xu *et al.*, 2005; Yu and Zheng, 2009). This kind of analysis must take into account variation in call parameters caused by factors

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such as air temperature (Chen *et al.*, 2012; Giacoma *et al.*, 1997; Navas and Bevier, 2001), male size and weight (Robertson, 1986; Giacoma *et al.*, 1997), and social interactions among individuals within the reproductive aggregation (Wells, 1988).

So far, the advertisement calls of more than 30 Chinese anurans have been reported, including *Microhyla ornata* (Jiang *et al.*, 1995; Wei *et al.*, 2013), *Rhacophorus dennysi* (Wang *et al.*, 2012), *D. melanostictus* (Wei *et al.*, 2012), *B. daunchina* (Chen *et al.*, 2011; Cui *et al.*, 2010, 2011, 2012), *Hoplobatrachus rugulosus* (Wei *et al.*, 2011), *Amolops wuyiensis* (Feng *et al.*, 2006; Shen *et al.*, 2008) and *Paa spinosa* (Chen *et al.*, 2012; Yu and Zheng, 2009). Previous research on Chinese anuran vocal behavior has used different methods to describe the acoustic structure of advertisement calls. For example, Jiang *et al.* (1995) used pulse duration, pulse rate, call duration and dominant frequency to describe the call characteristics of three frog species, but Yu and Zheng (2009) measured other parameters, including note number, note rate, note duration, number of formants (harmonics), and dominant frequency, in their analysis of the advertisement call of *P. spinosa*.

Babina adenopleura is a small-to medium-sized frog with a body length of 53–60 mm, and distributed in central and southern China. This species was first designated as *Rana adenopleura* by Boulenger (1909), but renamed as *B. adenopleura* according to the currently accepted taxonomy (Frost *et al.*, 2006). Its skin is blue-green or gray-brown with a pattern of dots on the dorsal surface (Boulenger, 1909). The species inhabits paddy fields, marshes, ditches, ponds, and hydrophytes-filled lakes at altitudes between 30 and 1 800 m. The breeding season of these frogs is prolonged and females begin to lay eggs in the second half of April; tadpoles of *B. adenopleura* in different development stage can be found between April to August; tadpoles during metamorphosis and young frogs after metamorphosis can be found in August; few tadpoles born too late begin metamorphosis after winter (Fei *et al.*, 2009).

B. adenopleura and *B. daunchina* are closely related and so similar in appearance that they were formerly classified as the same species; *B. daunchina* was considered to be the synonym of *B. adenopleura* and they were named *R. adenopleura* (Pope and Boring, 1940). These two related species all have paired internal pharyngeal vocal sacs which are diverticula of the mouth cavity and may serve as resonators, sound couplers, or acoustic radiators (Narins *et al.*, 2007). The most obvious differences between these two species are their spawning habits and calls (Fei *et al.*, 2009). Before pairing occurs, the male *B. daunchina* builds a mud-based burrow for mating and egg incubating (Liu, 1950; Ye *et al.*, 1993), and produce calls of varying lengths consisting of a series of "musical" note from inside or outside the burrows(Cui *et al.*, 2010; Chen *et al.*, 2011). But *B. adenopleura* does not have this unique behavior. Moreover, their calls can be easily distinguished by human ear. The acoustic structure of male *B. daunchina* calls and their correlation with air temperature and humidity has been described by Cui *et al.* (2010, 2011) and Chen *et al.* (2012). Males of this species use calls to advertise that they possess a nest to potential mates, thereby facilitating mate selection by females (Cui *et al.*, 2012). However, little is known about the call properties of *B. adenopleura* and previous research did not analyze its components in detail (Zhou *et al.*, 2014). The aims of this study were to describe the components of the advertisement call of *B. adenopleura*, determine the extent to which identifiable call features vary between calls and individuals, and compare the calls of this species with those of *B. daunchina*.

2. Materials and Methods

2.1 Recording of calls Male *B. adenopleura* advertisement calls were recorded in the Gutian Mountain National Nature Reserve in Quzhou City, Zhejiang Province, China (29.14° N, 118.05° E) during June and July 2012 and July 2013. Calls were recorded between 19:00 to 22:00 using Sony IC recorders (ICD-FX8) with an internal microphone. To avoid repeated recording, we found and identified subjects according to calls. Having identified a subject, the microphone was placed at about 50 cm from the subject. The distance was kept as constant as possible such that variation in microphone position. Calls of different males were recorded for 5–30 min. All calls were recorded from rice field with water, and frogs stayed along the ridge of the fields. After recording, six males (July 2013) were captured and body mass measured to the nearest 0.1g with an electric balance (Mettler Toledo International Inc., PL2001-L, Switzerland). Body size (snout-vent length) was measured to the nearest 0.01 mm using a digital caliper (Guilin Measuring and Cutting Tool Co. Ltd., China). Then the males were released into the site from which they were captured.

We used a Humidity Temperature Meter (Center Technology Group Company, CENTER 311, Taiwan, China) to measure air temperature (21–26 °C) and humidity (83%–94%). The value of temperature and humidity showed litter variation, so we did not analyse

whether these variables affected male calls.

2.2 Acoustic parameters Recording files were transferred with Cool Edit Pro2.0 (Syntrillium Software Corporation, USA) and saved as wav format. The calls of individual male frogs recorded in the field were usually contaminated acoustically by the calls of other males or other environmental sounds. Therefore, the calls of individual males were first edited from the original recordings using Cool Edit Pro2.0 software so that selected clean, uncontaminated, calls could be analyzed. More than ten calls in each call sequence within a sound file were analyzed. We successfully recorded the advertisement calls of 16 males and analyzed a total of 190 calls.

All sound files were digitized and analyzed using Praat (Boersma and Weeninkk, Version 5.3.32, University of Amsterdam) at a sampling frequency resolution of 44100 Hz and 16 bit resolution. Call parameters were defined and illustrated based on Pröhl (2003), Yu and Zheng (2009) and Chen *et al.* (2012). To describe clearly the acoustic structure of calls, the frequency band containing the most energy, or the single greatest amplitude, was defined as the dominant frequency (DF/Hz) (Figure 1C). The value of the fundamental frequency (Hz) was obtained by calculating the reciprocal of the period of the longest cycle (Chen *et al.*, 2011; Simmons and Ferragamo, 1993). Six other acoustic properties were measured: call duration (s), note number, note duration (s), note rate (notes/s), note interval (s) and number of harmonics. Yu and Zheng (2009) calculated the note rate with the equation: note rate = (note number – 1) / call duration. Since some calls have only one note in our study, we calculated note rate with the modified equation: note rate = note number / call duration. All spectral components were measured at the midpoint of notes. To provide a normative picture of frequency changes within and between the notes of advertisement calls, all harmonics (i.e., frequency components) of each note within all calls recorded for individual males were measured. Temporal and spectral parameters were measured for each of the first call notes because every call contained at least one note.

Subharmonics with durations shorter than the duration of the note could be identified in some call spectrograms (Figure 1B), and are reflected by the smaller peaks in the power spectrum (Figure 1C). Although the subharmonic bands were not always obvious and existed in only some calls, these features of *B. adenopleura* calls were considered pertinent to measure. Thus the number of subharmonics in note 1 and the duration of subharmonics in note 1 were measured.

To determine if the maximum value for the fundamental of call notes is related to the body mass and body size for individual males, we determined the highest fundamental frequency for all notes of the same call.

2.3 Analysis and statistics *B. adenopleura* calls consist of variable numbers of notes. To describe call characteristics, calls consisting of same numbers of notes were classified together for analysis. Differences in note duration and fundamental frequency between different notes were also analyzed with a one-way ANOVA. In the case of calls with just two notes, an Independent-Samples *t*-test was used to evaluate differences in note duration and fundamental frequency between notes. In the case of calls that consisted of variable numbers of notes, a one-way repeated measures ANOVA was used to evaluate the significance of differences in call duration, note rate, note interval, note duration, frequency bands and dominant frequency. Pearson's correlation analysis was used to detect whether the fundamental is related to the body mass and size of individual males.

Data were expressed as mean ± SD, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed with SPSS 13.0 for windows. All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (F-max test) prior to statistical analyses.

3. Results

3.1 Structure of *B. adenopleura* advertisement calls Male *B. adenopleura* advertisement calls have a distinctive structure. Advertisement calls (190 calls from 16 males) contained 1–5 notes (2.74 ± 0.92), each of approximately 0.2–0.9 s in duration. Three-note calls were the most common (70/190; 36.84%), followed by two-note calls (66/190; 34.74%), four-note calls (36/190; 18.95%), one-note calls (14/190; 7.37%), and five-note calls (4/190; 2.11%). There were silent intervals between the notes of a call (Figure 1A).

The mean note amplitude showed little variation within the calls of individual males (Figure 1A). To evaluate differences in note interval and note duration between different calls with variable numbers of notes, we measured and analyzed the mean values of these parameters. As shown in Table 1, call duration, note rate and note duration were significantly different between different calls with variable numbers of notes. Call duration was correlated with note number, while note interval and note duration were negatively correlated

with the note number. For calls with the same number of notes, except for 1 and 5 note calls, the first note in each call was significantly longer than the succeeding notes, whereas the succeeding notes differed little, if at all, in length (Table 2).

Each call note consisted of several frequency components that were not integer multiples of the lowest frequency band, indicating that the lowest frequency band (F_1) in each note was not the fundamental frequency which has been suppressed (Figure 1B, 1C). All frequency components of nine 3-note calls recorded from an individual male are shown in Table 3; frequency components were nearly integer multiples of 300 Hz. Fifth frequency bands (F_5) differ significantly between the first, second and third notes($F_{2, 24} = 3.450$, $P < 0.05$, Table 3). The number of subharmonics in note 1 is 3.088 ± 2.465 and the duration of submarmonics in note 1 is 0.053 ± 0.023 s.

The mean male body mass and size of *B. adenopleura* were 11.3 ± 1.0 g and 53.2 ± 3.1 mm, respectively (n = 6). Pearson's correlation analysis showed that the highest fundamental frequency was not positively correlated with either body mass ($r = 0.799$, $P > 0.05$, $n = 6$) or size ($r = 0.371$, $P > 0.05$, $n = 6$).

3.2 Frequency modulation patterns of calls Three different frequency modulation harmonic band (FM) patterns were observed. Six percent (6%) of call notes did not exhibit frequency modulation (Figure 2A). That is to say, their harmonics can be represented as horizontal

Table 1 Acoustic characteristics of male *B. adenopleura* advertisement calls.

Parameter	Call duration (s)	Note rate (notes/s)	Note interval (s)	Note duration (s)
1 note call (n = 14)	0.173 ± 0.031^{a}	5.957 ± 1.192^{b}		0.173 ± 0.031^{c}
2 notes call (n = 66)	0.454 ± 0.054^{b}	4.475 ± 0.575^{a}	0.141 ± 0.021^{c}	0.153 ± 0.022^{b}
3 notes call (n = 70)	0.716 ± 0.076^{c}	4.248 ± 0.533^{a}	0.134 ± 0.021^{b}	0.150 ± 0.015^{b}
4 notes call (n = 36)	0.906 ± 0.139^{d}	4.541 ± 0.832^{a}	0.120 ± 0.021^{b}	0.137 ± 0.020^{b}
5 notes call (n = 4)	0.900 ± 0.194^{d}	5.722 ± 1.017^{b}	0.090 ± 0.024^{a}	0.109 ± 0.020^{a}
Results of One Way ANOVA	$F_{4,185} = 281.210^{**}$	$F_{4,185} = 21.272^{**}$	$F_{3,172} = 13.273^{**}$	$F_{4,185} = 13.112^{**}$

Data are presented as mean ± SD, different superscripts in the same column indicate significant differences (SNK's test, α = 0.05, a < b < c < d). ** $P < 0.001$.

Table 2 Durations of successive notes in male *B. adenopleura* advertisement calls.

	1-note call (No. = 14)	2-note call (No. = 66)	3-note call (No. = 70)	4-note call (No. = 36)	5-note call (No. = 4)
1st note	0.173 ± 0.031	0.173 ± 0.025^{a}	0.180 ± 0.021^{a}	0.171 ± 0.031^{a}	0.138 ± 0.034
2nd note		0.133 ± 0.019^{b}	0.138 ± 0.013^{b}	0.131 ± 0.020^{b}	0.103 ± 0.016
3rd note			0.131 ± 0.012^{c}	0.126 ± 0.018^{b}	0.100 ± 0.018
4th note				0.121 ± 0.016^{b}	0.102 ± 0.016
5th note					0.102 ± 0.020
Results of One Way ANOVA			$F_{2,207} = 191.397^{**}$	$F_{3,140} = 38.948^{**}$	$F_{4,15} = 2.181^{NS}$

Data are presented as mean ± SD, different superscripts in the same column indicate significant differences (SNK's test, α = 0.05, a < b < c). NS not significant, ** $P < 0.001$.

Table 3 Mean frequencies of the first 11 partials for 3-note calls produced by a single male *B. adenopleura*.

	1st note (Hz)	2nd note (Hz)	3rd note (Hz)
F_1	567 ± 27 (9)	594 ± 33 (9)	597 ± 29 (9)
F_2	853 ± 53 (9)	896 ± 51 (9)	899 ± 43 (9)
F_3	1148 ± 65 (9)	1191 ± 68 (9)	1196 ± 58 (9)
F_4	1432 ± 77 (9)	1495 ± 79 (9)	1495 ± 72 (9)
F_5	1718 ± 86 (9)	1825 ± 108 (9)	1798 ± 75 (9)
F_6	2001 ± 104 (9)	2110 ± 117 (9)	2090 ± 105 (9)
F_7	2284 ± 114 (9)	2401 ± 130 (9)	2383 ± 111 (9)
F_8	2566 ± 141 (9)	2708 ± 149 (9)	2684 ± 140 (9)
F_9	2867 ± 167 (8)	2940 ± 158 (7)	2961 ± 153 (8)
F_{10}	3083 ± 137 (6)	3153 ± 11 (5)	3227 ± 128 (7)
F_{11}	3349 ± 139 (6)	3451 ± 37 (5)	3459 ± 20 (6)
F_{12}	3886 ± 41 (2)		3765 ± 0 (1)

Note: Call data from 3-note calls produced by a single male.

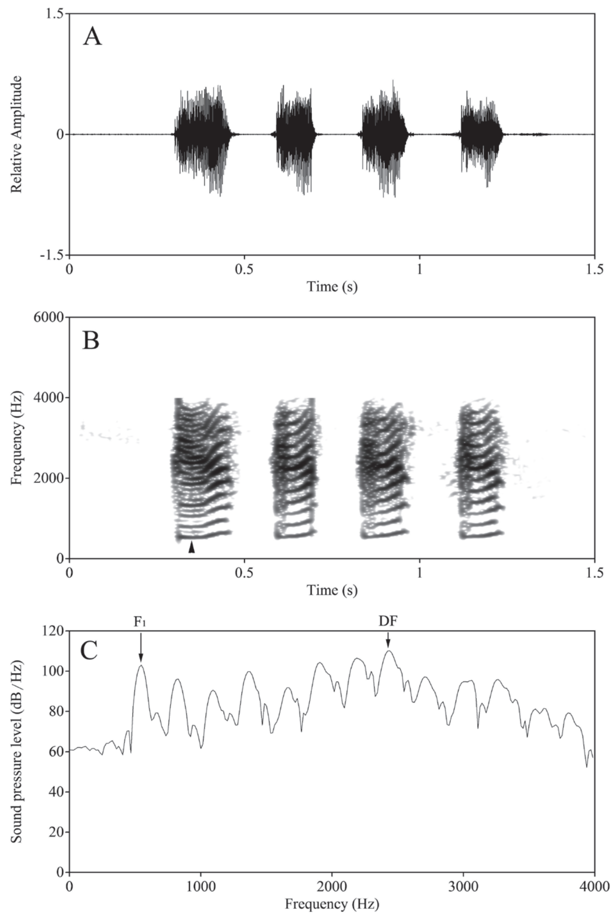

Figure 1 Waveforms (A), Spectrograms (B) and power spectrum (C) of advertisement call of male *B. adenopleura*. Subharmonic bands can be seen in the first note (indicated by an arrow); F_1 is lowest frequency band and DF is dominant frequency, the power spectrum was measured at the midpoint of the first note.

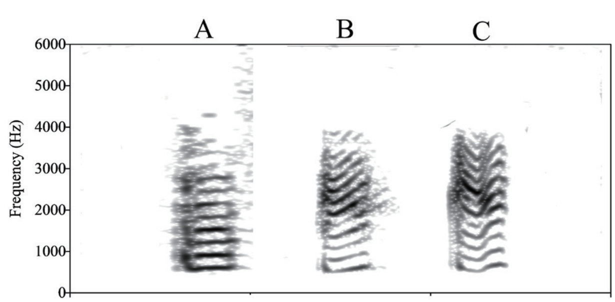

Figure 2 Spectrograms of advertisement calls of male *B. adenopleura* illustrating two different call frequency modulation patterns; (A) No obvious frequency modulation; (B) Upward frequency modulation pattern; (C) Concave frequency modulation pattern.

bands. However, two FM patterns were observed in other notes: (1) An upward FM pattern with ΔHz/ms of 0.882 ± 0.020 (Figure 2B); (2) A downward–upward FM pattern with ΔHz/ms of 0.537 ± 0.020 (Figure 2C). The upward FM pattern was a simple upward curve, whereas the downward–upward FM pattern was concave in shape; an upward curve followed by downward modulation to the baseline. Moreover, the first notes in multi-note calls were usually the note most likely to show the downward–upward FM pattern. Frequency modulation patterns varied for harmonic bands within single calls, and in some cases, varied from call to call.

3.3 Fundamental frequency increments between notes The fundamental frequency of successive notes varied between notes. This can also be seen in the results of the analysis of each note of 9 three note calls of an individual male (Table 3). To investigate this phenomenon further, we analyzed calls with the same number of notes (Table 4). The fundamental frequency of the first note within all multi-note calls was lowest, and increased substantially in the second notes. In 3 note ($F_{2,207} = 8$, $P < 0.001$) and 4 note ($F_{3,140} = 4$, $P < 0.05$) calls, the fundamental frequency of different notes was significantly different. The highest fundamental frequency of all multi-note calls (except 2 note calls) was usually found in the middle note. The first notes' fundamental frequency was lower than the last notes fundamental frequency in successive calls. This suggests that there an upper limit to fundamental frequency.

3.4 Dominant frequency variation The dominant frequency of calls was highly variable. For some calls, the lowest frequency band contained the highest energy, that is, the lowest frequency was the dominant frequency (Figure 3A), whereas in others calls the dominant frequency was much higher than the lowest harmonic. Analysis of the dominant frequency of first notes indicates that there were seven patterns of dominant frequency. The sixth harmonic was most common (located in 2165 ± 152 Hz section) (Figure 3D), followed by the eighth harmonic (located in 2466 ± 16 Hz section) (Figure 1C), fourth harmonic (located in 1712 ± 333 Hz section) (Figure 3C), seventh harmonic (located in 2269 ± 140 Hz section) (Figure 3E), first harmonic (located in 605 ± 65 Hz section) (Figure 3A), second harmonic (located in 919 ± 10 Hz section) (Figure 3B) and ninth harmonic (located in 2637 ± 21 Hz section) (Figure 3F). The value is significantly different ($F_{6,182} = 321.088$; $P < 0.001$).

4. Discussion

The advertisement calls of male anurans have two functions: attraction of conspecific females and male territorial advertisement (Wells, 1977; Tárano, 2001). Female anurans use species-specific male advertisement

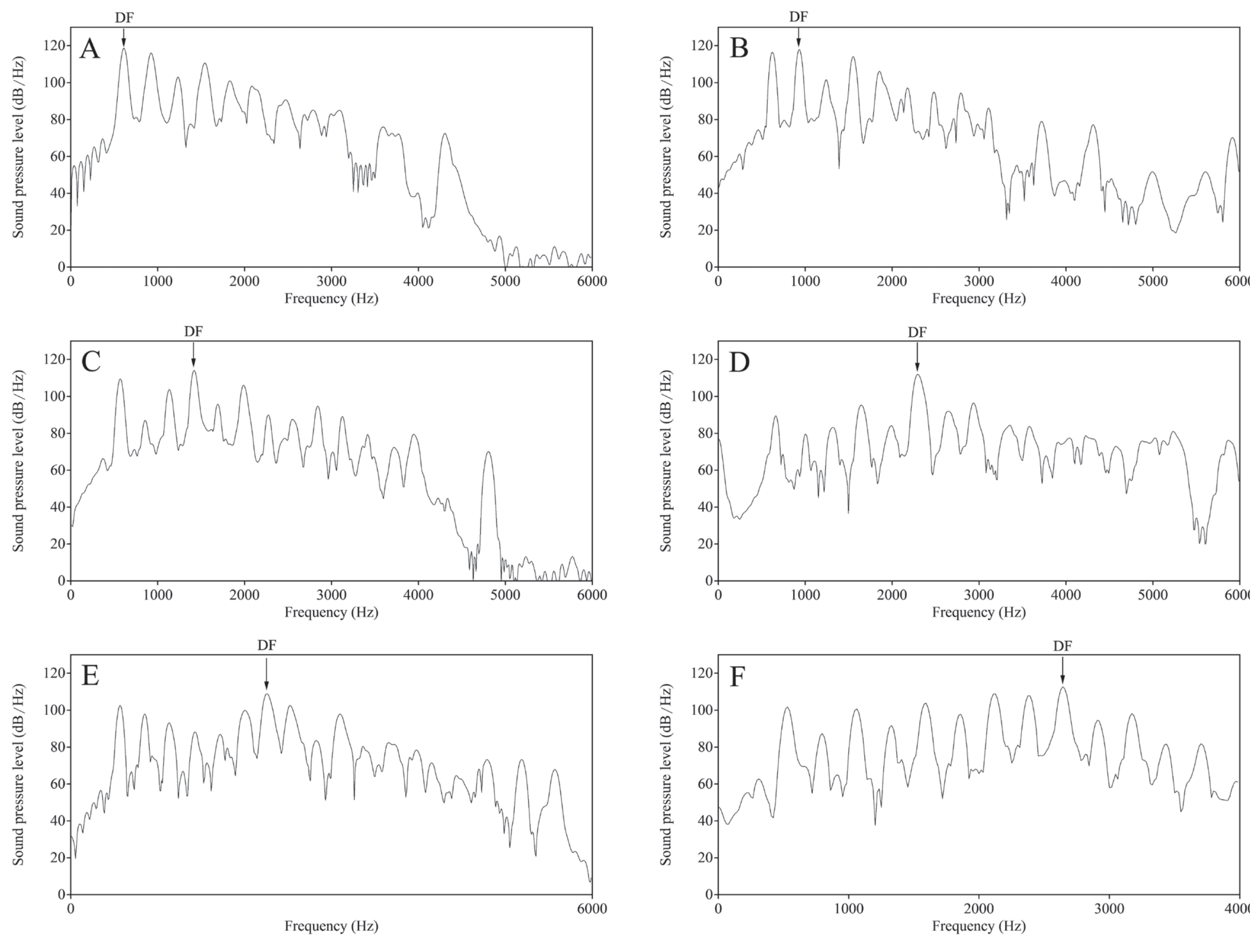

Figure 3 Five different power spectra of the advertisement calls of male *B. adenopleura* illustrating variation in the partial with the highest energy.

Table 4 Mean fundamental frequencies of male *B. adenopleura* advertisement calls of different length.

	1-note call (No. = 14)	2-note call (No. = 66)	3-note call (No. = 70)	4-note call (No. = 36)	5-note call (No. = 4)
1st note	295 ± 28	297 ± 25	287 ± 26^{a}	298 ± 27^{a}	309 ± 5
2nd note		313 ± 27	304 ± 28^{b}	316 ± 27^{b}	326 ± 15
3rd note			302 ± 27^{b}	316 ± 26^{b}	324 ± 8
4th note				312 ± 27^{b}	327 ± 10
5th note					319 ± 2
Results of One Way ANOVA			$F_{2,207} = 8^{**}$	$F_{3,140} = 4^{*}$	$F_{4,15} = 2^{NS}$

Data are presented as mean ± SD, different superscripts in the same column indicate significant differences (SNK's test, α = 0.05, a < b < c). NS not significant, * $P < 0.05$, ** $P < 0.001$.

calls to identify conspecific males (McClelland *et al.*, 1996; Lea *et al.*, 2002; Krishna and Bosch, 2007), and often to decide which male to mate with (Gerhardt, 1994; Pröhl, 2003; Yu and Zheng, 2009; Cui *et al.*, 2012).

We found that the advertisement calls of male *B. adenopleura* were typically comprised of 1–5 notes, the mean amplitude of which did not vary. Each note consisted of a stack of frequency bands and there were silent intervals between notes (Figure 1A). Except for 1- and 5-note calls, the first note in each call was significantly longer than the succeeding notes, and note duration subsequently decreased sequentially (Table 2). Note interval was negatively correlated with note number (Table 1). Male anurans typically spend a significant amount of time calling, for example, Yu *et al.* (2008) found that calling comprised 20% of the time budgets of individual male *P. spinosa* during the breeding season. It's possible, therefore, that male *B. adenopleura* most

commonly give three-note calls because these are relatively energy efficient to produce. This is because, for multi-note calls, the longer the inter-note interval, the smaller the energy required to produce the call (Jiang *et al.*, 2002). Yu *et al.* (2008) speculated that the absence of silent intervals between notes in the calls of *P.spinosa* may reduce energy consumption.

The fundamental frequency is a missing spectral component of *B. adenopleura* advertisement calls; a similar phenomenon occurs in the green frog (*R. clamitans*) (Bee *et al.*, 2001). Among anuran amphibians (frogs and toads), fundamental frequency depends on the shape and mass of the laryngeal apparatus, which is related to overall body size (Martin, 1972). Although the body mass and size of individual subjects was not positively correlated with the highest fundamental frequency in this study, there is much evidence suggesting this relation which supports the idea that the call characters of anuran males can provide information about body size (Robertson, 1986; Wang *et al.*, 2012). Therefore, the female can evaluate the potential male's quality on the basis of the acoustic features of male communication signals (Morris and Yoon, 1989). Furthermore, the fundamental frequency increased substantially in the second notes, but changed little in the following notes. This also indicates that increases in the fundamental frequency are limited by the size of the vocal sac in *B. adenopleura* as is the case for the túngara frog (*Physalaemus pustulosus*) (Ryan, 1983). More study is needed to resolve this question.

The dominant frequency of each note varied to some degree and was not associated with any particular harmonic band, i.e., the harmonic with the highest energy varied. We found seven patterns of dominant frequency in the first note of calls; these were in the first, second, fourth, sixth, seventh, eighth and ninth harmonic, respectively. This is a very interesting phenomenon. As is the case for the fundamental frequency, the dominant frequency of the call is negatively correlated with size in many species (Bee *et al.*, 2001; Sullivan and Wagner 1988; Wang *et al.*, 2012), due to morphological constraints on the sound producing apparatus (Martin, 1972). Because of this relationship, males of many species use the dominant frequency of the call to assess the size of an opponent (Davies and Halliday 1978; Arak 1983; Robertson 1986). However, there is also empirical evidence that the dominant frequency of frog calls may be plastic (Grafe, 1995; Lopez *et al.*, 1988; Wagner, 1992), especially during social interactions among competing males. For example, male white-lipped frogs (*Leptodactylus albilabris*) increased or decreased the dominant frequency of their calls to match that of a nearby male (Lopez *et al.*, 1988). At the time our recordings were made, a large number of male *B. adenopleura* called in the same rice field. Furthermore, the dominant frequency of the call from the same frog was also changed. So males may vary the dominant frequency of their calls for competing with other males (Wei *et al.*, 2011). Playback tests are needed to explore this phenomenon further.

We found many similarities between the acoustic characteristics of the calls of male *B. adenopleura* from the Gutian Mountain National Nature Reserve and those of male *B. daunchina* from the Emei mountain area of Sichuan province (Chen *et al.*, 2012). For example, the advertisement calls of both species were comprised of multiple notes with several frequency components, the dominant frequency of each note varied to some degree, and fundamental frequency changes between notes and calls displayed prominent frequency modulation characteristics. However, there were many differences between the calls of these two species. The male advertisement calls of *B. daunchina* contained more notes than those of *B. adenopleura*; although a few calls with 2 or 9 notes were recorded, most *B. daunchina* advertisement calls contained 3–8 notes (4.86 ± 1.31). In contrast, male *B. adenopleura* advertisement calls contained 1–5 notes (2.74 ± 0.92). Because the advertisement calls of these species have different note numbers, we could only compare the call duration, note number and acoustic parameters of the first note of each species. The duration of the first note was longer in *B. daunchina* (0.23 ± 0.05 s) than in *B. adenopleura* (0.17 ± 0.03 s) but the number of harmonics in the first note of *B. adenopleura* (4.97 ± 1.96) calls was greater than in *B. daunchina* (11.12 ± 1.96), and the dominant frequency of the first note were higher in *B. adenopleura* (2018.05 ± 568.18 Hz) than in *B. daunchina* (845.09 ± 393.23 Hz) but the fundamental frequency of the first note was lower in in *B. adenopleura* (293.91 ± 26.00 Hz) than in *B. daunchina* (462.19 ± 85.24 Hz). Whereas the lowest frequency band of *B. adenopleura* call notes was not the fundamental frequency which has been suppressed, and subharmonics were only present in some calls, the fundamental frequency of call notes is normally not suppressed in *B. dauncnina* call notes and subharmonics were present in most call notes.

In summary, male *B. adenopleura* calls are spectrally and temporally complex. Most call notes exhibited frequency modulation; fundamental frequency changes between notes and variation in the dominant frequency

of each note. This may be to reduce energy consumption or because of anatomical constraints. The acoustic characteristics we identified are useful for distinguishing *B. adenopleura* from other species such as the morphologically very similar *B. daunchina*.

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